

# Piperidine Alkaloids in Sitka Spruce with Varying Levels of Resistance to White Pine Weevil (Coleoptera: Curculionidae)

ELIZABETH A. GERSON AND RICK G. KELSEY<sup>1</sup>

USDA Forest Service, PNW Research Station, 3200 SW Jefferson Way, Corvallis, OR 97331

J. Econ. Entomol. 95(3): 608–613 (2002)

**ABSTRACT** Our objective was to evaluate piperidine alkaloids as potential resistance factors in Sitka spruce, *Picea sitchensis* (Bong.) Carr, at risk to attack by white pine weevils, *Pissodes strobi* (Peck). We sampled 72 seedlings in each of two replicated field trials in the Oregon Coast Range. The seedlings were grown from open-pollinated seeds of putatively “resistant” or “susceptible” off-site parental sources. Alkaloid concentrations in bark and foliage were measured in previously unattacked trees at the time of weevil host selection. Leader mortality was evaluated in the fall to gauge actual resistance in the sample trees. Five families had  $\leq 25\%$  topkill and seven sustained  $>50\%$  topkill. Alkaloid concentrations differed significantly among families, but the major alkaloids did not appear to be functionally linked with topkill or useful indicators of resistance. However, our study design did not address all potential resistance mechanisms. Therefore, before concluding that Sitka spruce alkaloids have no influence on white pine weevils, complementary laboratory and field experiments are needed.

**KEY WORDS** *Pissodes strobi*, *Picea sitchensis*, leader mortality, host plant resistance, alkaloids

THE WHITE PINE weevil, *Pissodes strobi* (Peck), is a serious deterrent to regeneration of Sitka spruce, *Picea sitchensis* (Bong.) Carr, on the Pacific slope of the Oregon Coast Range (Harris 1990). Risk of weevil damage to Sitka spruce is especially high in the southern extent of its range, which includes western Oregon and a narrow coastal band in California (Harris 1990, Peterson et al. 1997). Weevils deposit eggs in the terminal shoot of young trees, and ensuing larvae consume the phloem, often causing leader mortality and stem deformities which restrict height growth and wood quality (Silver 1968, Hamid et al. 1995). A recent survey of 41 Sitka spruce stands in the northern Oregon Coast Range revealed an average 2.8 defects per tree, 34% of which were forks or major crooks in the stem (Lysak 2001). Renewed interest in growing Sitka spruce in its historic range along the Oregon coast, where it attains maximum development (Peterson et al. 1997), has emerged as an unprecedented outbreak of Swiss needle cast now seriously affects Douglas-fir plantations (Filip et al. 2000). In addition, coastal Sitka spruce rain forest is relatively rare and unique among the world's temperate forests, providing further incentive for successful management of this resource.

To test the viability of growing offsite, weevil-resistant Sitka spruce in the Oregon Coast Range, two trial plantations were established by the Oregon Department of Forestry (ODF) in 1994. We took advantage of these progeny trials to conduct a comparative study of piperidine alkaloids in resistant and suscep-

tible spruce. Spruce defensive chemistry, with emphasis on terpenes and resin acids, has been studied in detail for possible involvement in weevil host selection and resistance (e.g., Alfaro et al. 1980; Brooks et al. 1987; Hrutford and Gara 1989; Manville et al. 1994; Tomlin and Borden 1997a, 1997b; Tomlin et al. 1997; Nault et al. 1999), but the alkaloids constitute a unique class of secondary compounds that had not been examined. Sitka spruce harbors a suite of 2,6-disubstituted piperidines in needles, branch, and root tissues (Stermitz et al. 1994, 2000). Although concentrations (dry weight basis) are highly variable, branch tissue tends to have more alkaloids than foliage (Stermitz et al. 1994, 2000). Preliminary analyses indicated higher alkaloid concentrations in bark (outer bark and phloem) than in sapwood from branch tips. Weevil adults, as well as their larvae, feed on phloem of terminal leaders (Silver 1968), hence the alkaloids are in a position to function as antifeedants or toxicants. Adult female weevils may also feed on spruce foliage before selecting trees for ovipositing (Silver 1968), so the potential exists for antibiosis by the foliar alkaloids as well. Resistance appears to result, at least in part, from unidentified water-soluble constituents of spruce bark that cause hormonal changes which inhibit egg production in female weevils (Leal et al. 1997; Sahota et al. 1998, 2001). We have found the piperidine alkaloids to be water-soluble (unpublished data) and constitutive in nature (Gerson and Kelsey 1998). Also, a mixture of pine alkaloids has been shown to be teratogenic in a frog embryo test (Tawara et al. 1993). Thus, we had reason to suspect that Sitka spruce

<sup>1</sup> E-mail: rkelsey@fs.fed.us.

Table 1. Family classification of sample trees from two progeny plantations, seed source, and current-year leader mortality

| OR Dept. of Forestry<br>Family no. (putative<br>resistance class) <sup>a</sup> | Seed source <sup>b</sup> | Incidence of topkill |                | Predicted % likelihood of topkill <sup>c</sup><br>(95% CI) |                  |
|--|--------------------------|----------------------|----------------|--|------------------|
|  |                          | Hamlet site          | S. Coal site   | Hamlet site  | S. Coal site     |
|  |                          | (n = 6)              | (n = 6)        |  |                  |
| 1025 (R)   | Qualicum Beach # 3, V.I. | 0                    | 0              | 0 (not estim.) <sup>d</sup>                                | 0 (not estim.)   |
| 1023 (R)   | Qualicum Beach # 1, V.I. | 1                    | 1              | 22.0 (5.6–57.3)  | 11.4 (2.5–39.1)  |
| 1027 (R)   | Ladysmith # 1, V.I.      | 2                    | 0              | 22.0 (5.6–57.3)  | 11.4 (2.5–39.1)  |
| 041 (S)  | Astoria District, Oregon | 1                    | 1              | 22.0 (5.6–57.3)  | 11.4 (2.5–39.1)  |
| 1036 (R)   | Gillies Bay # 4, V.I.    | 1                    | 2              | 32.2 (10.9–65.0)   | 17.8 (5.0–46.9)  |
| 1394 (R)   | Knight Inlet? V.I.       | 5                    | 1              | 59.7 (30.5–83.4)   | 40.3 (16.7–69.5) |
| 5003 (S)   | Q.C.I. (b)               | 4                    | 2              | 59.7 (30.5–83.4)   | 40.3 (16.7–69.5) |
| 1032 (R)   | Mill Bay # 1, V.I.       | 5                    | 2              | 67.8 (37.8–87.9)   | 48.9 (22.0–76.5) |
| 5001 (S)   | Q.C.I. (a)               | 4                    | 3              | 67.8 (37.8–87.9)   | 48.9 (22.0–76.5) |
| 5005 (S)   | Q.C.I. (d)               | 4                    | 4              | 75.3 (45.4–91.8)   | 58.1 (28.1–83.1) |
| 5006 (S)   | Q.C.I. (e)               | 4                    | 4              | 75.3 (45.4–91.8)   | 58.1 (28.1–83.1) |
| 5004 (S)   | Q.C.I. (c)               | 4                    | 4 <sup>e</sup> | 79.8 (49.1–94.2)   | 64.3 (30.9–87.9) |

From logistic regression analysis: the predicted likelihood of leader mortality and 95% confidence intervals, depending on site and family class.

<sup>a</sup> A priori classification based on parent trees: (R), “resistant”; (S), “susceptible.”

<sup>b</sup> All parent trees located in B.C. except 041; Q.C.I., Queen Charlotte Islands; V.I., Vancouver Island.

<sup>c</sup> Predicted values are influenced by overall site effects, so predictions at the family level do not necessarily mirror the topkill rates.

<sup>d</sup> Confidence interval not estimable because of all-zero data.

<sup>e</sup> (n = 5).

alkaloids could be involved in resistance to *Pissodes strobi*.

Weevil attacks were low in the Oregon progeny trials the first 3 yr after seedlings were planted, but in the fourth year (1998), attack rates among susceptible progeny were as high as 40 and 55%, respectively, at the two plantations (ODF, Forest Management Division). Populations were expected to be large in the spring of 1999, but enough unattacked saplings remained in the progeny plantations to form a sample pool for a prospective, observational study of Sitka spruce with varying levels of resistance. At this stage of weevil population growth, trees are likely to be exposed to fairly uniform, heavy weevil pressure (He and Alfaro 1997). We sampled bark and foliage in late spring when weevils typically select host trees, then later the same year we surveyed each sample tree to assess leader mortality. Open-pollinated progeny of resistant parent trees inherently vary in actual resistance (Manville et al. 1994, Nault et al. 1999), so we chose not to rely on simply categorizing sample trees as “resistant” or “susceptible” based on parentage. Although Sitka spruce leaders may be fed upon, and oviposited in, they can ultimately survive to produce an intact, straight stem for the tree. Therefore, we did not attempt to evaluate intermediate levels or stages of weevil attack or damage; instead, we defined weevil resistance solely in terms of leader mortality (topkill).

With alkaloid concentrations at the time of host selection modeled as potential explanatory variables, and topkill as the subsequent response variable, we used logistic regression to evaluate whether the alkaloids are likely to be involved in weevil resistance. Secondly, any alkaloids found to be positively or negatively correlated with subsequent leader mortality could provide useful markers for weevil-resistant genotypes, and could aid reforestation efforts with Sitka spruce.

## Materials and Methods

**Sampling Procedure.** Tissue samples were collected from two Sitka spruce plantations installed in the Oregon Coast Range by the ODF for a weevil resistance trial. The “Hamlet” test site (UTM Zone 10; 5 077 209 m N, 441 190 m E; 16.3 km inland) in Clatsop State Forest, and the “Southern Coal” test site (UTM Zone 10; 5 066 185 m N, 434 590 m E; 5.1 km inland) in Tillamook State Forest, were planted in March of 1994 with 2-yr-old seedlings. Thus, seeds for resistant and susceptible trees came from different geographical locations, but the sample trees themselves were grown in a common environment. Putatively “resistant” open-pollinated progeny of parent trees in British Columbia, Canada, were planted along with “susceptible” progeny from Queen Charlotte Islands, BC, and local Oregon stock of unknown resistance. For the current study, we randomly selected six of the 16 “resistant” families in the trial, and six of eight “susceptible” families. The open-pollinated progeny of a common parent tree or seed source are referred to as “family” and represent a group of sample trees with similar genotype. The parent tree locations for the 12 families sampled in our study are listed in Table 1.

At each test site, six trees were randomly selected from each of the 12 families for a total of 144 sample trees. Trees with evidence of previous weevil attack, i.e., trees with dead leaders, stem deformities, weevil brood gallery scars, or resin from feeding or oviposition holes, were excluded from the sample pool. Damaged trees were excluded to preclude the possibility our chemical analyses might reflect induced chemical changes. Tissue samples were collected 18 May 1999 at Southern Coal, and 21 May 1999 at Hamlet. At this time, female weevils were expected to be actively selecting spruce leaders for oviposition (Overhulser and Gara 1975). On 24 August and 1 September 1999, we visually examined each sample tree at Southern

Coal and Hamlet, respectively, and recorded leader mortality.

**Tissue Handling Procedure.** Two lateral branches from the top whorl of each sample tree were clipped and stored in paper bags at ambient temperature for alkaloid analysis (Gerson and Kelsey 1999a). Substitution of lateral branches for leaders was done to avoid negative impacts on the ODF progeny trial. A preliminary comparison of alkaloid concentrations in leaders and laterals from three naturally regenerated saplings at Southern Coal showed no differences in paired *t*-tests, although *cis*-pinidinol concentrations were two-fold higher in terminals from two of the three trees.

Upon return to the laboratory, sapwood was removed from the branches. Weevil larvae feed in the phloem, and preliminary extractions of sapwood indicated it contained a very small percentage of whole stem alkaloids, therefore it was discarded. Buds also were discarded because weevils do not use this tissue. Needles and bark (specifically, outer bark and phloem) were dried 72 h at 70°C, separated, and ground to pass a 20-mesh screen. Ground tissue was sealed in plastic bags at -20°C until extraction.

**Alkaloid Analysis.** Half-gram samples were extracted according to the optimized solid-phase partitioning procedure detailed in Gerson and Kelsey (1999a). All known piperidine alkaloids were quantified using Hewlett-Packard (now Agilent Technologies, Wilmington, DE) instruments including a 5890/II gas chromatograph (GC) fitted with a 30 m by 0.25 mm DB-1 capillary column (0.25  $\mu$ m film thickness, J & W Scientific, Agilent Technologies), a 5970 mass selective detector, a 7673 automatic sampler, and ChemStation software. One microliter of extract was introduced into a 250°C injection port with a 5:1 split, and the oven temperature was ramped from 85 to 135°C at 5°C per minute. The system was calibrated daily using *cis*-dihdropinidine and *cis*-pinidinol as external standards, and 2-ethylpiperidine as an internal standard.

**Statistical Analyses.** The potential for alkaloids to explain susceptibility to weevil topkill was evaluated using logistic regression (PROC GENMOD, SAS Institute 1996). Sample tree topkill was modeled as a dependent, binary response to the alkaloid concentrations, with site included as a blocking variable. Drop-in-deviance tests based on chi-square probabilities (Ramsey and Schafer 1997) were used to determine whether individual or total alkaloids were significantly linked with topkill. Separate regressions were done for bark and needle tissues. To determine whether the likelihood of leader mortality differed among family classifications, another logistic regression, with site as a blocking variable, was run to generate 95% confidence intervals.

Multivariate analysis of variance (MANOVA was used to test the effect of family classification on concentrations of alkaloids. Because of the large number of compounds measured, separate MANOVAs were done for bark alkaloids and needle alkaloids. Each MANOVA modeled the full suite of alkaloids present in the tissue class as multivariate responses to a linear function of site and family effects. The response vari-

ables were transformed to natural logarithms to meet assumptions of normality and homoscedacity. Where MANOVA test statistics (PROC GLM, SAS Institute 1990) indicated very low probability of no overall family effect ( $P < 0.001$ ), the individual compounds were tested by analysis of variance (ANOVA) for differences among families. If the null hypotheses for family effects in these ANOVAs were improbable, then family means were compared. This tiered approach to testing for differences when many response variables (chemical components) are measured should help protect against compounding overall experimental error (Scheiner 1993). Least-squares means (LS means) for families and limits defined by one standard error for these means, were backtransformed for presentation.

## Results

The following piperidine alkaloids were identified in extracts of the sample tissues according to published mass spectra (Hart et al. 1967, Schneider et al. 1991, Tawara et al. 1993, Todd et al. 1995): 1,6-imine [2-methyl-6-propyl-1,6-piperideine]; epidihdropinidine, (2-methyl-6-propylpiperidine); pinidinol, [2-methyl-6-(2-hydroxypropyl)piperidine]; 1,2-dehydropinidinol, [2-methyl-6-(2-hydroxypropyl)-1,2-piperideine]; pinidinone, [2-methyl-6-(2-oxopropyl)piperidine]; and euphococcinine, (1-methyl-9-*nor*-3-granatanone). *Cis*-pinidinol was the predominant alkaloid in bark tissue with concentrations ranging from zero to 2,760  $\mu$ g/g dw. *Trans*-pinidinol was detected infrequently at low concentrations. Epidihdropinidine and euphococcinine were detected commonly at concentrations <100  $\mu$ g/g dw. The 1,6-imine, pinidinone, and 1,2-dehydropinidinol were detected less frequently at concentrations below 40, 80, and 150  $\mu$ g/g dw, respectively. Total alkaloid concentrations in bark ranged widely from 21 to 3,216  $\mu$ g/g dw. In needle tissue, epidihdropinidine and euphococcinine were the dominant alkaloids with concentrations as high as 440 and 810  $\mu$ g/g dw, respectively. The 1,6-imine, pinidinone, and *cis*-pinidinol occurred less frequently at concentrations below 100, 110, and 180  $\mu$ g/g dw, respectively. Total alkaloid concentrations in needles ranged from 70 to 1,215  $\mu$ g/g dw.

From the logistic regressions we found that none of the alkaloids, alone or in total, in bark or in foliage, were good predictors of leader mortality (all  $P > 0.40$ ). Family classification was a significant predictor for topkill ( $P = 0.004$ ), however, 95% confidence intervals for the probabilities were large (Table 1). For example at the Hamlet site, sample trees in resistant family 1023 had a 6–57% likelihood of being topkilled, whereas the range for trees in susceptible family 5004 was 49–94%. Families 1023, 1025, 1027, 1036, and the local Oregon family 041 had consistently lower incidence of leader mortality in sample trees at both sites (Table 1). Two of the putatively “resistant” Canadian families (1032 and 1394) at the Hamlet site sustained heavy topkill during the season in which we sampled. All putatively “susceptible” Canadian families (5000-series) had high rates of topkill.

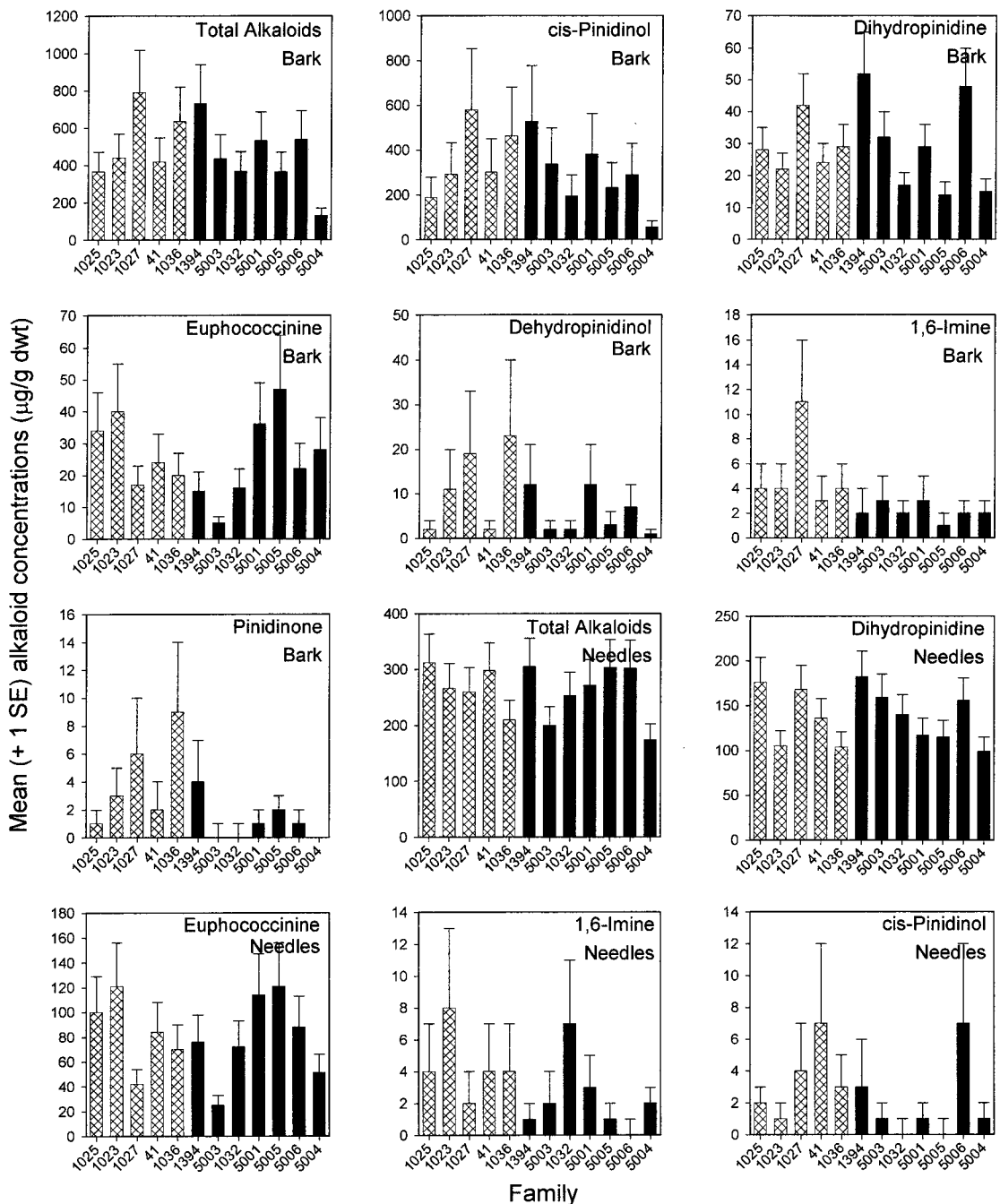


Fig. 1. Mean  $\pm$  1 SE concentrations ( $\mu\text{g/g dry weight}$ ) of alkaloids in 1-yr-old bark and needles from 12 families of Sitka spruce. Means represent 12 sample trees from two sites. Hatched bars designate families with topkill in  $\leq 25\%$  of sample trees; black bars indicate families with topkill rates  $\geq 50\%$ .

In the MANOVAs, overall tests of family effects were highly significant (all  $P \leq 0.002$ ). For every compound, differences among families were indicated (all  $P \leq 0.087$ , generally  $\leq 0.01$ ). Family means ( $\pm 1$  SE) are presented for the alkaloids in Fig. 1. The site factor generally was significant ( $P < 0.05$ ) in the MANOVAs. Total alkaloid concentrations in nee-

dles and bark were higher at the Southern Coal site (Table 2).

### Discussion

Sitka spruce families had clear differences in their alkaloid concentrations. Expression of conifer alka-



Table 2. ANOVA results for comparison of total alkaloids in foliage and bark of Sitka spruce in two Oregon progeny trials

| Response variable | Tissue  | Site    | LS mean <sup>a</sup><br>( $\mu\text{g/g}$ dwt) | 95% CI<br>( $\mu\text{g/g}$ dwt) |
|-------------------|---------|---------|--|----------------------------------|
| Total alkaloids   | Foliage | Hamlet  | 224  | 197–252                          |
|                   |         | S. Coal | 301  | 265–340                          |
|                   | Bark    | Hamlet  | 391  | 316–475                          |
|                   |         | S. Coal | 499  | 402–607                          |

<sup>a</sup>  $n = 72$  sample trees per site.

loids appears to be under significant genetic control, with high variability among provenances, and among open-pollinated progeny within provenance (unpublished data for ponderosa pine in a common-garden genetics study). Exceptionally low concentrations of *cis*-pinidinol and euphococcine in bark of susceptible families, such as 5004 and 5003, respectively, may warrant further study. Although it is a minor compound, the highest concentrations of 1,6-imine consistently occurred in resistant families, especially 1027 (see Fig. 1). Alkaloid bioactivity is highly variable and may depend on minor structural differences (Saunders et al. 1991, Brown and Trigo 1995). It is entirely possible some of the spruce piperidines are toxic at very small concentrations, while others are neutral. Therefore, the potential efficacy of minor compounds should not be underestimated.

The difference in alkaloid concentrations between the two study sites was an interesting finding. Concentrations of alkaloids change significantly in young pine needles as they mature, but are thought to remain relatively stable in older foliage (Gerson and Kelsey 1998). We sampled year-old foliage and bark from Sitka spruce, therefore the site differences in their alkaloids were more likely the result of environmental than phenological differences. Alkaloids in pines are known to vary substantially among sites (Gerson and Kelsey 1998) and can be influenced by nitrogen availability (Gerson and Kelsey 1999b). Similarly, Wainhouse et al. (1998) have shown that other secondary compounds of Sitka spruce are responsive to alterations in resource availability.

Before conducting this study, we postulated that alkaloids might be involved in Sitka spruce resistance to *Pissodes strobi*. However, our data failed to link spring alkaloid concentrations with spruce leader mortality, and consequently they do not appear to be functionally involved, or have any value as markers for weevil resistance. But, our study design does not address the possibilities that changed alkaloid concentrations later in the year could affect weevil larvae as they develop (e.g., an induced alkaloid response), or that other mediating factors obscured a weak alkaloid effect (rendering any alkaloid effect moot). Whether the conifer alkaloids are 'inducible' remains to be determined. Laboratory bioassays, including the minor spruce alkaloids, to directly test for physiological effects on weevil reproduction would be informative. Complementary laboratory and field studies would be necessary to conclusively rule out a role for alkaloids in weevil resistance.

## Acknowledgments

We are grateful to Dave Overhulser, Tona Mortensen, and Steve Skinner (Oregon Department of Forestry) for providing access, background information, and field assistance on the Sitka spruce progeny trials. Manuela Huso (Oregon State University) provided substantial consultations on the statistical analysis of the data. We also appreciate the helpful comments of Joerg Bohlmann (University of British Columbia) and Darrell W. Ross (Oregon State University) regarding the preparation of the manuscript.

## References Cited

- Alfaro, R. I., H. D. Pierce, Jr., J. H. Borden, and A. C. Oehlschlager. 1980. Role of volatile and nonvolatile components of Sitka spruce bark as feeding stimulants for *Pissodes strobi* Peck (Coleoptera: Curculionidae). *Can. J. Zool.* 58: 626–632.
- Brooks, J. E., J. H. Borden, and H. D. Pierce, Jr. 1987. Foliar and cortical monoterpenes in Sitka spruce: potential indicators of resistance to the white pine weevil, *Pissodes strobi* Peck (Coleoptera: Curculionidae). *Can. J. For. Res.* 17: 740–745.
- Brown, K. S., Jr., and J. R. Trigo. 1995. The ecological activity of alkaloids, pp. 227–354. In G. A. Cordell (ed.), *The alkaloids: chemistry and pharmacology*, vol. 47. Academic, San Diego, CA.
- Filip, G., A. Kanaskie, K. Kavanaugh, G. Johnson, R. Johnson, and D. Maguire. 2000. Silviculture and Swiss needle cast: research and development recommendations. Research Contribution 30. Forestry Research Laboratory, Oregon State University, Corvallis, OR.
- Gerson, E. A., and R. G. Kelsey. 1998. Variation of piperidine alkaloids in ponderosa (*Pinus ponderosa*) and lodgepole pine (*P. contorta*) foliage from central Oregon. *J. Chem. Ecol.* 24: 815–827.
- Gerson, E. A., and R. G. Kelsey. 1999a. Foliar storage and extraction methods for quantitative analysis of piperidine alkaloids from ponderosa pine (*Pinus ponderosa*). *Phytochem. Anal.* 10: 322–327.
- Gerson, E. A., and R. G. Kelsey. 1999b. Piperidine alkaloids in nitrogen fertilized *Pinus ponderosa*. *J. Chem. Ecol.* 25: 2027–2039.
- Hamid, A., T. M. Odell, and S. Katovich. 1995. White pine weevil. USDA For. Serv. For. Insect Dis. Leaflet 21.
- Harris, A. S. 1990. *Picea sitchensis* (Bong.) Carr., pp. 260–267. In R. M. Burns and B. H. Honkala (eds.), *Silvics of North America*, vol. 1. Conifers. USDA For. Serv. Agric. Handb. 654.
- Hart, N. K., S. R. Johns, and J. A. Lamberton. 1967. (+)-9-aza-1-methylbicyclo[3,3,1] nonane-3-one, a new alkaloid from *Euphorbia atoto* Forst. Aust. *J. Chem.* 20: 561–563.
- He, F., and R. I. Alfaro. 1997. White pine weevil (Coleoptera: Curculionidae) attack on white spruce: spatial and temporal patterns. *Environ. Entomol.* 26: 888–895.
- Hrutford, B. F., and R. I. Gara. 1989. The terpene complement of slow and fast growing Sitka spruce terminals as related to *Pissodes strobi* (Peck) (Col., Curculionidae) host selection. *J. Appl. Entomol.* 108: 21–23.
- Leal, I., E. E. White, T. S. Sahota, and J. F. Manville. 1997. Differential expression of the vitellogenin gene in the spruce terminal weevil feeding on resistant versus susceptible host trees. *Insect Biochem. Molec. Biol.* 27: 569–575.
- Lysak, T. 2001. Hazard rating system for spruce weevil (*Pissodes strobi*) in sitka spruce in the northern Oregon coast range. M.S. thesis, Oregon State University, Corvallis.

- Manville, J. F., J. Nault, E. von Rudloff, A. Yanchuk, and G. K. Kiss. 1994. Spruce terpenes: expression and weevil resistance, pp. 203–217. In R. I. Alfaro, G. Kiss, and R. G. Fraser (eds.), *The white pine weevil: biology, damage and management*. Proceedings, Symposium 19–21 January 1994, Richmond, BC. FRDA Report No. 226. Canadian Forest Service and British Columbia Ministry of Forests, Victoria, BC.
- Nault, J. R., J. F. Manville, and T. S. Sahota. 1999. Spruce terpenes: expression and weevil resistance. *Can. J. For. Res.* 29: 761–767.
- Overhulser, D. L., and R. I. Gara. 1975. Spring flight and adult activity of the white pine weevil, *Pissodes strobi* (Coleoptera: Curculionidae), on Sitka spruce in western Washington. *Can. Entomol.* 107: 251–256.
- Peterson, E. B., N. M. Peterson, G. F. Weetman, and P. J. Martin. 1997. Ecology and management of Sitka spruce, emphasizing its natural range in British Columbia. UBC, Vancouver, BC.
- Ramsey, F. L., and D. W. Schafer. 1997. *The statistical sleuth: a course in methods of data analysis*. Duxbury, Belmont, CA.
- Sahota, T. S., J. F. Manville, F. G. Peet, E. White, A. Ibaraki, and J. R. Nault. 1998. Resistance against white pine weevil: effects on weevil reproduction and host finding. *Can. Entomol.* 130: 337–347.
- Sahota, T. S., J. F. Manville, J. Hollman, I. Leal, A. Ibaraki, and E. White. 2001. Resistance against *Pissodes strobi* (Coleoptera: Curculionidae) in severed leaders and in a water-soluble bark extract of *Picea sitchensis* (Pinaceae): evidence for a post-ingestive mode of action. *Can. Entomol.* 133: 315–323.
- SAS Institute. 1990. *SAS/STAT user's guide*, version 6, 4th ed. SAS Institute, Cary, NC.
- SAS Institute. 1996. *SAS/STAT software: changes and enhancements through release 6.11*. SAS Institute, Cary, NC.
- Saunders, J. A., N. R. O'Neill, and J. T. Romeo. 1991. Alkaloid chemistry and feeding specificity of insect herbivores, pp. 151–172. In S. W. Pelletier (ed.), *Alkaloids: chemical and biological perspectives*, vol. 8. Springer, New York.
- Scheiner, S. M. 1993. MANOVA: multiple response variables and multispecies interactions, pp. 94–112. In S. M. Scheiner and J. Gurevitch (eds.), *Design and analysis of ecological experiments*. Chapman & Hall, New York.
- Schneider, M. J., J. A. Montali, D. Hazen, and C. E. Stanton. 1991. Alkaloids of *Picea*. *J. Nat. Prod.* 54: 905–909.
- Silver, G. T. 1968. Studies on the Sitka spruce weevil, *Pissodes sitchensis*, in British Columbia. *Can. Entomol.* 100: 93–110.
- Stermitz, F. R., J. N. Tawara, M. Boeckl, M. Pomeroy, T. A. Foderaro, and F. G. Todd. 1994. Piperidine alkaloid content of *Picea* (spruce) and *Pinus* (pine). *Phytochemistry* 35: 951–953.
- Stermitz, F. R., C. D. Kamm, and J. N. Tawara. 2000. Piperidine alkaloids of spruce (*Picea*) and fir (*Abies*) species. *Biochem. Syst. Ecol.* 28: 177–181.
- Tawara, J. N., A. Blokhin, T. A. Foderaro, and F. R. Stermitz. 1993. Toxic piperidine alkaloids from pine (*Pinus*) and spruce (*Picea*) trees. New structures and a biosynthetic hypothesis. *J. Org. Chem.* 58: 4813–4818.
- Todd, F. G., F. R. Stermitz, and A. V. Blohkin. 1995. Piperidine alkaloid content of *Picea pungens* (Colorado blue spruce). *Phytochemistry* 40: 401–406.
- Tomlin, E. S., and J. H. Borden. 1997a. Thin bark and high density of outer resin ducts: interrelated resistance traits in Sitka spruce against the white pine weevil (Coleoptera: Curculionidae). *J. Econ. Entomol.* 90: 235–239.
- Tomlin, E. S., and J. H. Borden. 1997b. Multicomponent index for evaluating resistance by Sitka spruce to the white pine weevil (Coleoptera: Curculionidae). *J. Econ. Entomol.* 90: 704–714.
- Tomlin, E. S., J. H. Borden, and H. D. Pierce. 1997. Relationship between volatile foliar terpenes and resistance of Sitka spruce to the white pine weevil. *For. Sci.* 43: 501–508.
- Wainhouse, D., R. Ashburner, E. Ward, and J. Rose. 1998. The effect of variation in light and nitrogen on growth and defence in young Sitka spruce. *Funct. Ecol.* 12: 561–572.

Received for publication 4 October 2001; accepted 15 December 2001.